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SAGA and a novel *Drosophila* export complex anchor efficient transcription and mRNA export to NPC

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SAGA/TFTC-type multiprotein complexes play important roles in the regulation of transcription. We have investigated the importance of the nuclear positioning of a gene, its transcription and the consequent export of the nascent mRNA. We show that E(y)2 is a subunit of the SAGA/TFTC-type histone acetyl transferase complex in *Drosophila* and that E(y)2 concentrates at the nuclear periphery. We demonstrate an interaction between E(y)2 and the nuclear pore complex (NPC) and show that SAGA/TFTC also contacts the NPC at the nuclear periphery. E(y)2 forms also a complex with X-linked male sterile 2 (Xmas-2) to regulate mRNA transport both in normal conditions and after heat shock. Importantly, E(y)2 and Xmas-2 knockdown decreases the contact between the heat-shock protein 70 (*hsp70*) gene loci and the nuclear envelope before and after activation and interferes with transcription. Thus, E(y)2 and Xmas-2 together with SAGA/TFTC function in the anchoring of a subset of transcription sites to the NPCs to achieve efficient transcription and mRNA export.

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Introduction

The nuclear periphery has been initially suggested to be a transcriptionally repressive nuclear subcompartment. The role of the nuclear periphery as a repressive compartment has come from studies carried out in *Saccharomyces cerevisiae* where it has been shown that telomeres form clusters at the nuclear periphery, which lead to enrichment of Sir proteins that are involved in gene silencing (Cockell and Gasser, 1999). Similarly, several inactive human genes were shown to associate with the nuclear periphery and with the perinuclear heterochromatin, whereas in their actively transcribed states these gene loci preferentially associated with euchromatin in the nuclear interior (Zink *et al*, 2004). The nuclear lamina at the nuclear periphery binds to chromatin and is thought to function in its organization. A genome-wide search for B-type lamin-associated genes in *Drosophila* indicated that associated genes are transcriptionally silent, lack active histone marks and are widely spaced (Pickersgill *et al*, 2006). Interestingly, it has recently been demonstrated in yeast that the physical tethering of a genomic loci to the nuclear pore complex (NPC) can dramatically alter its activity and thus, has identified the NPC as a new, integral participant of gene expression (Ishii *et al*, 2002; Schmid *et al*, 2006). In addition, in yeast, transcriptional activation of the *GAL1*, *INO1* and *HXK1* genes results in their dynamic association with nuclear pore proteins and relocation to the nuclear periphery (Brickner and Walter, 2004; Casolari *et al*, 2004; Cabal *et al*, 2006; Taddei *et al*, 2006; Akhtar and Gasser, 2007).

Considerable evidence indicates that the different steps involved in the synthesis, biogenesis and export of mRNAs are tightly linked to each other. The general RNA polymerase II (Pol II) transcription factors and especially Pol II play a central role in all these events, as they mediate the recruitment to nascent mRNAs of factors involved in 5′ capping, splicing, 3′ end formation and mRNA export (Dantonel *et al*, 1997; Manley, 2002; Proudfoot *et al*, 2002; Vinciguerra and Stutz, 2004). Recent studies identified additional mRNA export factors that link Pol II transcription and mRNA export. Yeast Sac3 was identified in a screen for mutations synthetically lethal with component of the TREX (transcription and export) mRNA export complex (Fischer *et al*, 2002; Reed and Cheng, 2005). Sac3p forms a complex with Thp1, (Gallardo and Aguilera, 2001) and deletion of either Thp1p or Sac3 results in poly(A)+ RNA export defects. Thp1–Sac3 may be recruited to mRNPs at an early stage of transcription and subsequently function in docking mRNP complexes to the nuclear face of the NPC (Fischer *et al*, 2002). Interestingly, Sac3 was also found at the cytoplasmic fibrils of NPCs (Lei *et al*, 2003).

E(y)2 was identified in a *Drosophila* genetic screen that was set up to search for genes that play a role in enhancer–promoter communication (Georgiev, 1994). *E(y)2* encodes a

small, evolutionarily conserved *Drosophila* protein of 101 amino acid, which is present in all tissues, is localized to the nucleus and is associated with numerous sites along the entire length of the salivary gland polytene chromosomes, suggesting that it plays a role in the regulation of gene expression (Georgieva *et al*, 2001). Both genetic and biochemical experiments demonstrated an interaction between E(y)2 and TAF9 (formerly E(y)1), a component of both the general transcription factor, TFIID and the *Drosophila* SAGA/TFTC-type histone acetyl transferase (HAT) coactivator complex involved in chromatin remodelling and transcription initiation of a subset of Pol II genes (Kusch *et al*, 2003; Muratoglu *et al*, 2003; Pankotai *et al*, 2005). The yeast homologue of E(y)2 was later called Sus1 and shown to be a component of both the SAGA and the Sac3–Thp1 mRNA export complexes (Rodriguez-Navarro *et al*, 2004), suggesting a connection between SAGA-dependent gene expression and the mRNA export machinery in yeast. Sus1 is a nuclear protein that concentrates at NPCs, and its localization at the nuclear membrane depends on Sac3. Consistent with its binding to SAGA, Sus1 is recruited to SAGA-dependent genes during transcription initiation (Kohler *et al*, 2006).

Here, we have investigated the importance of the nuclear positioning of a gene, its transcription and the consequent export of the nascent mRNA in a *metazoan* organism. We show that E(y)2, the *Drosophila* homologue of yeast Sus1, is a component of the SAGA/TFTC-type complex. We demonstrate an interaction between E(y)2 and the NPC and show that SAGA/TFTC also contact the NPC in the cells. E(y)2 forms a complex with X-linked male sterile 2 (Xmas-2) protein, the homologue of yeast Sac3, and both proteins are required for mRNA transport. Importantly, E(y)2 and Xmas-2 knockdown reduces the six heat-shock protein 70 (*hsp70*) gene loci in contact with the nuclear envelope before and after activation and interferes with *hsp70* transcription. Thus, E(y)2–Xmas-2-containing novel *Drosophila* anchoring and mRNA export complex (AMEX) together with the E(y)2-containing dSAGA/TFTC complex participate in the anchoring of a subset of transcription sites to the NPC basket to achieve efficient transcription and mRNA export.

Results

E(y)2 is a bona fide subunit of the Drosophila SAGA/TFTC complex and colocalizes with GCN5 on polytene chromosomes

We have tested whether the *Drosophila* (d) homologue of yeast Sus1, called E(y)2 (Georgieva *et al*, 2001), is a subunit of the dSAGA/TFTC complex. To this end, *Drosophila* embryo nuclear extract was treated with RNase and DNase and proteins were immunoprecipitated with specific polyclonal antibodies raised against E(y)2 or dGCN5 (Supplementary Figure 1), the latter being a known subunit of the dSAGA/TFTC complex. The anti-E(y)2 immunoprecipitation (IP) co-purified known dSAGA/TFTC subunits, such as dTRRAP, dGCN5, dADA2b and dTAF10 (Figure 1A, lane 2; Kusch *et al*, 2003; Muratoglu *et al*, 2003), whereas the control IP using the pre-immune sera did not (lane 3). Similarly, the anti-GCN5 IP co-purified E(y)2 together with other known dSAGA/TFTC subunits (lane 5). These results together indicate that E(y)2 is a *bona fide* subunit of the *Drosophila* SAGA/TFTC complex.

To confirm that E(y)2 also associates with the dSAGA/dTFTC complex in *Drosophila* cells, we have carried out salivary glands polytene chromosome staining using anti-GCN5 and anti-E(y)2 antibodies. Anti-E(y)2 antibody staining revealed that E(y)2 associates with a large number of loci (Figure 1B) and that many of these loci were also stained by the anti-dGCN5 antibody (see merges in Figure 1B), indicating a colocalization between GCN5 and E(y)2.

The E(y)2-containing SAGA/TFTC-type complex is in contact with the nuclear pore complex

Next, we analyzed the localization of E(y)2 in *Drosophila* Schneider (S2) cells. We have observed that E(y)2 is localized to dots in the nucleus (Figure 2A and B, panel a), which appear similar to those obtained with anti-GCN5 and anti-ADA2b antibodies (Figure 2A and B, panels b and i). This result further suggests that GCN5, ADA2b and E(y)2 may colocalize in common nuclear structures, which may be different from those labelled by an anti-TAF1 antibody (Figure 2A and B, panel m). However, in contrast to GCN5

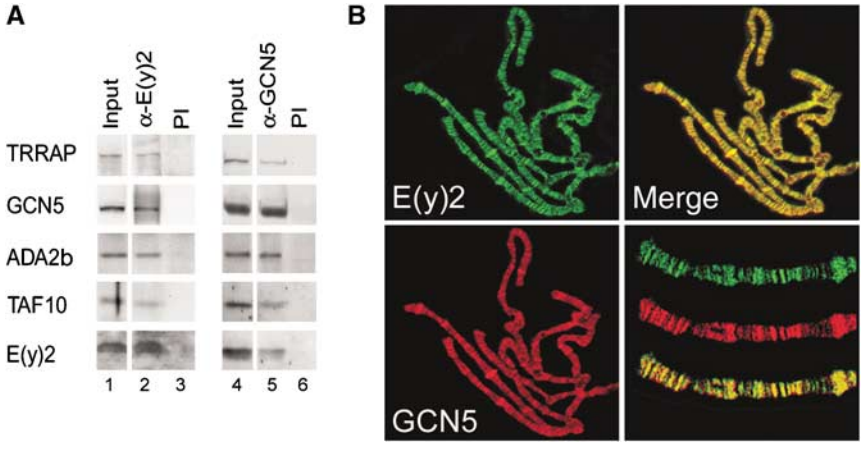

Figure 1 E(y)2 is a *bona fide* subunit of the *Drosophila* SAGA/TFTC complex. (**A**) The *Drosophila* embryo nuclear extract was immunoprecipitated with antibodies raised against GCN5, E(y)2 or a rabbit preimmune serum (PI). Input nuclear fraction (7.5%) (Input), and protein A-Sepharose-antibody-bound proteins (15%) (IP), were resolved by SDS–PAGE. Blots were revealed using antibodies raised against TRRAP, GCN5, ADA2b, TAF10 and E(y)2. (**B**) E(y)2 colocalizes with GCN5 at many sites on polytene chromosomes of *Drosophila*. Whole chromosomes, enlarged fragments as well as the merged images are shown.

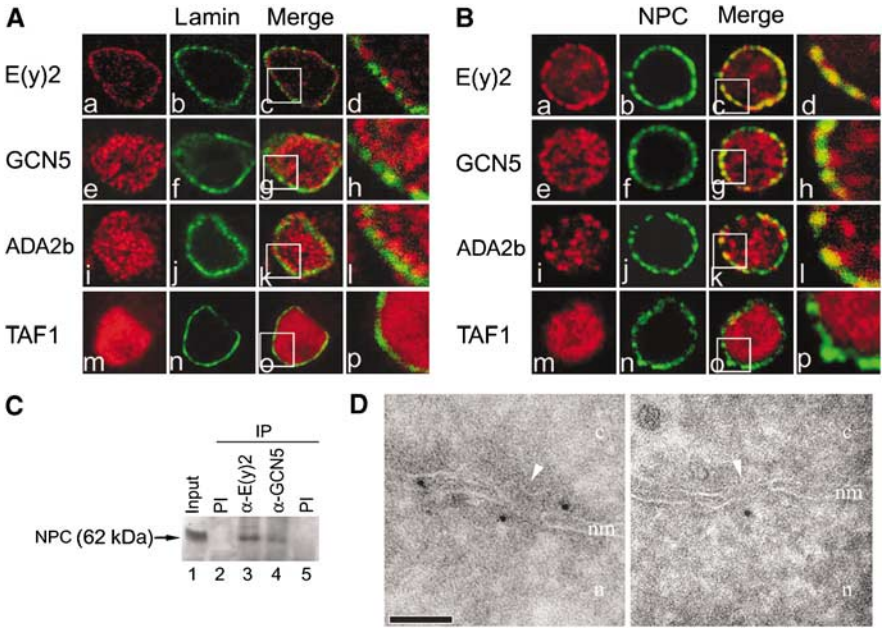

Figure 2 E(y)2 is localized to nuclear periphery, is in contact with the NPC and is also present in dots in the nucleus. (**A**) The immunostaining of *Drosophila* S2 cells with antibodies against E(y)2, GCN5, ADA2b or TAF1 (in red), an antibody against lamin (in green) (magnification, $\times 1000$). (**B**) Immunofluorescent staining of *Drosophila* S2 cells with antibodies against E(y)2, GCN5, ADA2b or TAF1 (in red), an antibody against NPC (Mab414) (in green) (magnification, $\times 1000$). (**C**) *Drosophila* embryo nuclear extract was immunoprecipitated with antibodies raised against E(y)2, GCN5 or with preimmune serum (PI). The input nuclear fraction (7.5%) (Input) and the protein A-Sepharose-antibody-bound proteins (15%) (IP) were resolved by SDS–PAGE. Blots were revealed with Mab414 with identifies nucleoporin p62 on western blots. (**D**) Gallery of electron micrographs showing ultrathin cryo sections of *Drosophila* S2 cells labelled with purified E(y)2-specific antibody and revealed with Protein A gold. Gold particles, which can be distinguished as small black dots, are preferentially located in the vicinity of the nuclear membrane (nm) and appear often associated with the NPC, shown by white arrow heads. The nuclear and cytoplasmic sides in the cells are labelled (n) and (c), respectively. Scale bar: 100 nm.

and ADA2B labelling, E(y)2 localized also to nuclear structures that were in, or close to, the nuclear envelope (Figure 2A and B, panel a). These data suggest that E(y)2 has dual or multiple roles in the cells.

In agreement with the finding that E(y)2 is a subunit of the transcriptional coactivator SAGA/TFTC complex, E(y)2 staining did not colocalize with that of nuclear lamin staining, which is supposed to label nuclear membrane regions associated with transcriptionally silent genes (Figure 2A; Pickersgill *et al*, 2006). As the E(y)2 staining was highly enriched at the nuclear periphery, next we analyzed whether E(y)2 would colocalize with proteins of the NPC. When proteins of the NPC and E(y)2 were stained, colocalization was observed at the nuclear periphery (Figure 2B, panels c and d). We further have carried out anti-E(y)2 immunopurification using S2 cell nuclear extracts treated with RNase and DNase and verified whether NPC components would associate with E(y)2. The anti-E(y)2 antibody co-purified NPC components with E(y)2 (Figure 2C, lane 3), indicating that E(y)2-containing complexes can be found in contact with NPC components in the cells. As E(y)2 is a component of the dSAGA/TFTC complex, we also tested whether there is a colocalization between NPC and SAGA/TFTC subunits such as GCN5. The anti-GCN5 antibody co-immunoprecipitated NPC components (Figure 2C, lane 4), however, to a lesser extent than the anti-E(y)2 IP, suggesting that a fraction of the cellular SAGA/TFTC complex can also contact the NPCs. When typical SAGA/TFTC subunits, such as GCN5 and ADA2b, were co-stained with NPC components (Figure B, panels g–i and k), a colocalization between the two complexes could also be detected in some regions close to the nuclear envelope. In good agreement, certain of the nuclear dots labelled by the GCN5 antibodies by immunofluorescence colocalize with E(y)2 close to the nuclear membrane (Supplementary Figure 1). These results together suggest that E(y)2 is present in two nuclear subcompartments, one that colocalizes with components of the NPC and second, in the dots inside in the nucleus. Moreover, in regions close to the nuclear envelope, dSAGA/TFTC subunits localize also close to NPCs.

Ultrastructural localization of E(y)2 by electron microscopy

To localize the E(y)2 protein at the ultrastructural level, cryo-fixed *Drosophila* S2 cells were cryo sectioned, labelled with the purified anti-E(y)2 antibody, revealed with gold-labelled protein A and observed by electron microscopy. The labelling was preferentially localized in the vicinity of the inner nuclear membrane (Figure 2D) and a frequent, although not exclusive, colocalization of E(y)2 with the nuclear pores complexes (Figure 2D) was detected, confirming the biochemical and the low resolution immunofluorescence data (see above).

Drosophila E(y)2 forms an endogenous complex with X-linked male sterile-2 protein

As our cellular localization studies suggested that E(y)2 is present possibly in two cellular structures, we carried out a yeast two-hybrid screen to identify further E(y)2 interacting partners (data not shown). In this analysis, using the *Drosophila* cDNA encoding the full-length E(y)2 as a bait, one of the isolated positive cDNA clones encoded

the *Drosophila* Xmas-2 (GenBank: AF216664) protein (Figure 3A). Xmas-2 at that point was a protein of unknown function. Interestingly, a blast search indicated that the 130 kDa Xmas-2 protein has a significant homology to the yeast Sac3p protein, playing a role in mRNA export, throughout an about a 200 amino-acid region corresponding to GANP domain of Sac3 (Supplementary Figure 2). The fragment of Xmas-2 (from amino acids 755-1370) that was found to interact with E(y)2 is distinct from the GANP domain, however, it also contains several conserved regions with Sac3p.

Next, we wanted to investigate the interaction between E(y)2 and Xmas-2 in *Drosophila* cells. To this end, the *Drosophila* embryo nuclear extract treated with RNase and DNase was immunoprecipitated with specific polyclonal antibodies raised against either E(y)2 or Xmas-2 (Supplementary Figure 1). The anti-E(y)2 IP co-purified Xmas-2 (Figure 3B, lane 4) and *vice versa* the anti-Xmas-2 IP co-precipitated E(y)2 (lane 6), whereas the IPs using the preimmune sera were negative for both proteins (lanes 5 and 7). Note, however, that not all cellular Xmas-2 co-precipitated with E(y)2, and *vice versa* not all E(y)2 co-immunoprecipitated with Xmas-2 (Figure 3B, compare lanes 2 and 3 with 4 and 6), suggesting that in the cells both E(y)2 and Xmas-2 are present either as free proteins or as subunits of other complexes. This is in good agreement with the finding that E(y)2 is present also in the dSAGA/TFTC complex.

To confirm that E(y)2 also associates with Xmas-2 *in vivo*, we have carried out salivary glands polytene chromosome staining using either anti-Xmas-2 or anti-E(y)2 antibodies. Anti-Xmas-2 antibody staining revealed that Xmas-2 associates with a large number of loci (Figure 3C) and that many of these loci were also stained by the anti-E(y)2 antibody (see merges in Figure 3C), indicating an *in vivo* colocalization between Xmas-2 and E(y)2. Next, we tested the colocalization of Xmas-2 and E(y)2 in S2 cells and also compared the localization of Xmas-2 to that of lamin and NPCs (Figure 3E). These results indicated that Xmas-2 and E(y)2 colocalize at many, but not all sites on the nuclear periphery (Figure 3E, panels c and d). This is in good agreement with the co-immunoprecipitation data (Figure 3B) and further shows that E(y)2 and Xmas-2 are often present together at the nuclear periphery, but can also be found in different other complexes. Moreover, the localization of Xmas-2 on the nuclear periphery of S2 cells also often coincides with that of NPCs, but not with that of lamin.

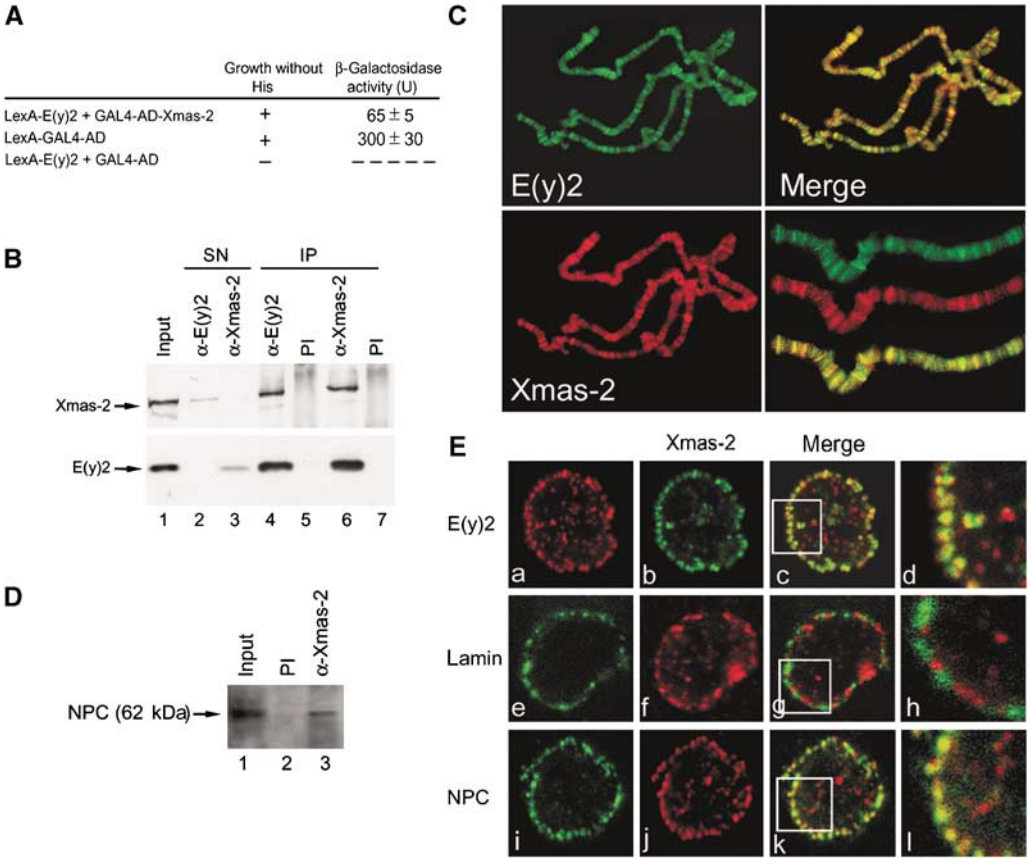

Figure 3 E(y)2 interacts with Xmas-2 and both proteins colocalize with NPC on the nuclear periphery. (**A**) The results of interaction of E(y)2 fused to the C-terminus of LexA with Xmas-2 (amino acids 755–1370) fused GAL4 activation domain (AD) in yeast two-hybrid assay. The activation of *HIS3* and *LacZ* reporter genes were measured. β-galactosidase activity (*U*) was determined using the following formula: $U = 1000 \times OD_{578}/(t \times 0.5 \times OD_{600})$, where *t* is the incubation time (in min). (**B**) Endogenous E(y)2 interacts with Xmas-2 *in vivo*. The *Drosophila* embryo nuclear extract was immunoprecipitated with antibodies raised against E(y)2 or Xmas-2, or preimmune serum (PI). A 7.5% portion of the input nuclear fraction (Input), 7.5% of the supernatant of the IPs (SN) and 20% of the protein A-Sepharose-bound proteins (IP), were resolved by SDS–PAGE. Blots were revealed using antibodies raised against E(y)2 and Xmas-2. (**C**) E(y)2 colocalizes with Xmas-2 at many sites on polytene chromosomes. Whole chromosomes, enlarged fragments as well as the merged images are shown. (**D**) *Drosophila* embryo nuclear extract was immunoprecipitated with polyclonal antibodies against Xmas-2, or with preimmune serum (PI). Input nuclear fraction (7.5%) (Input) and protein A-Sepharose-antibody-bound proteins (15%) (IP) were resolved by SDS–PAGE. Blots were revealed with Mab414. (**E**) The co-immunostaining of *Drosophila* S2 cells with antibodies raised against Xmas-2, E(y)2, lamin and Mab414 (magnification, × 1000).

The interaction between Xmas-2 and the NPC was also demonstrated in a *Drosophila* embryo nuclear extract treated with RNase and DNase from which the anti-Xmas-2 antibody co-immunoprecipitated NPC components (Figure 3D).

We then wondered whether Xmas-2 has an influence on E(y)2 localization. RNA interference (RNAi) knockdown of Xmas-2 in *Drosophila* cells (Figure 4A) led to a redistribution of E(y)2 in the nuclei and importantly, the amount of E(y)2 associated with the nuclear envelope strongly decreased (Supplementary Figure 2). These results together indicate that (i) E(y)2 and Xmas-2 form a complex; (ii) that E(y)2 is present in at least two different endogenous complexes, one together with Xmas-2 (and possibly other proteins), and the other in the dSAGA/TFTC complex; and (iii) and that both E(y)2 and Xmas-2 often colocalize with NPCs, but not with lamin. In addition, the localization of E(y)2 to the nuclear periphery requires Xmas-2.

Both E(y)2 and Xmas-2 are required for efficient mRNA transport

To investigate the role of *Drosophila* Xmas-2 and E(y)2 in mRNA export, we have carried out RNAi experiments to knock down either E(y)2 or Xmas-2 expression in S2 cells. The efficiency of the RNAi was verified by either RT–PCR or by western blot analysis (Supplementary Figure 3; Figure 4A). In these RNAi experiments, the localization of poly(A)+ RNA was assessed with a Cy3-labelled oligo(dT) probe. In wild-type and in control RNAi-treated cells (performed using a fragment of pBSK vector) the majority of the poly(A)+ RNA accumulates in the cytoplasm (Figure 4B, upper panel; Supplementary Figure 4). In contrast, when either E(y)2 or Xmas-2 expression was knocked down for 5 days, the poly(A)+ RNA accumulated in the nucleus (Figure 4B; Supplementary Figure 4).

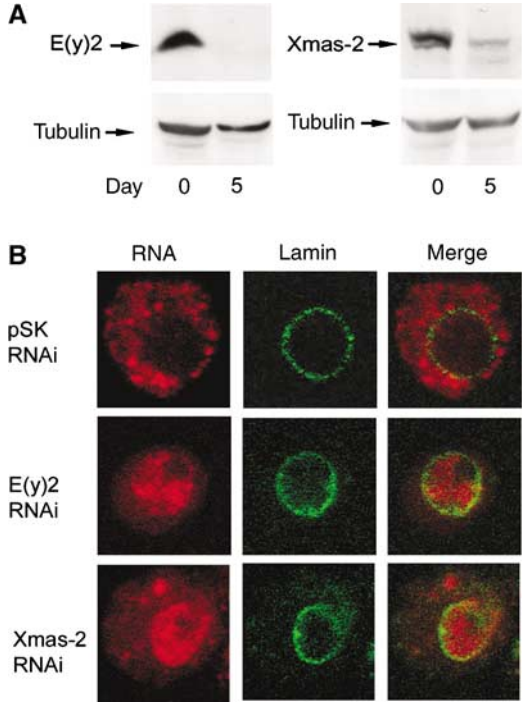

Figure 4 E(y)2 and Xmas-2 are required for mRNA export from the nucleus. (**A**) The efficiency of E(y)2 or Xmas-2 knockdown was tested by western blot analysis. E(y)2 and Xmas-2 protein expression present in wild-type S2 cells (0) or in S2 cells after 5 days of RNAi treatment (5) were analyzed. Tubulin was used as a loading control. (**B**) E(y)2 and Xmas-2 are required for poly(A)+ RNA export from the nucleus. RNAi was performed using either dsRNA corresponding to a fragment of pSK II vector as a control or the *E(y)2* or the *Xmas-2* cDNAs. RNA FISH was carried out using a Cy3-labelled oligo(dT) probe to identify poly(A)+ RNA. Nuclear envelope is stained with lamin. Representative examples of cells are shown (magnification, × 1000).

Next we analyzed whether the downregulation of either E(y)2 or Xmas-2 also influences mRNA transport at the individual transcript level. To this end, we have either carried our RNA fluorescent *in situ* hybridization (RNA FISH) experiments using probes hybridizing with the *hsp70* transcripts or carried out RT–PCR experiment on nuclear RNA samples using oligonucleotides amplifying RNAs transcribed either from the *hsp70*, the *actin* or the *trf2* genes. As the *hsp70* genes can be synchronously activated in *Drosophila* cells by heat shock (HS) (Zhimulev, 1999), the effect of the E(y)2 or Xmas-2 RNAi on the *hsp70* transcripts were measured under both non-HS (NHS) and HS conditions. Using these two different methods, we have observed that the knockdown of either E(y)2 or Xmas-2 RNAi under the different conditions blocked the nuclear export of the tested transcripts, as an increase in the nuclear accumulation of the corresponding RNAs was observed (Figure 5A and B; Supplementary Figure 5). Defining the nuclear amount of the *hsp70*, the *actin* or the *trf2* transcripts as one arbitrary unit under control RNAi treatment, we observed a 4- to 10-fold increase of the *hsp70* transcripts when either E(y)2 or Xmas-2 were knocked down independent of the HS (Figure 5). Similarly, following either E(y)2 or Xmas-2 RNAi treatment the *actin* and the *trf2* transcripts levels increased also two-threefold in the nucleus (Figure 5B). Importantly, the RNAi treatment did not influence the length and the quality of these transcripts when tested by northern blot (Figure 6D). Our data together demonstrate that E(y)2 and Xmas-2 are required for efficient mRNA transport and thus, they are novel components of the *Drosophila* mRNA export machinery.

E(y)2 and Xmas-2 participate in the anchoring of the hsp70 loci to the nuclear envelope independent of activation

Next we wanted to investigate whether E(y)2 and Xmas-2 would regulate the positioning of different gene loci and their regulation. To this end, we examined the localization of the dSAGA/TFTC-dependent *hsp70* gene cluster, containing six almost identical copies located at chromosomal loci 87A and 87B (Zhimulev, 1999). We chose to analyze the *hsp70* gene clusters, because dSAGA/TFTC was shown to be present at *hsp70* promoters (Lebedeva *et al*, 2005). When the nuclear localization of the wild-type endogenous *hsp70* genes was determined by DNA FISH under NHS conditions, following observations were made: (i) the six *hsp70* genes localized non-randomly to the nuclear periphery (in 65% of the cases); and (ii) they stayed mostly together in one territory (see WT NHS *hsp70* in Figure 6A and B). To quantify the localization of the *hsp70* DNA FISH signal, the nuclear space was divided into three concentric zones of equal surface and the signal was mapped as described earlier (Hediger *et al*, 2004; Taddei *et al*, 2004). Interestingly, when the endogenous *hsp70* gene transcription was activated by HS, the localization of the

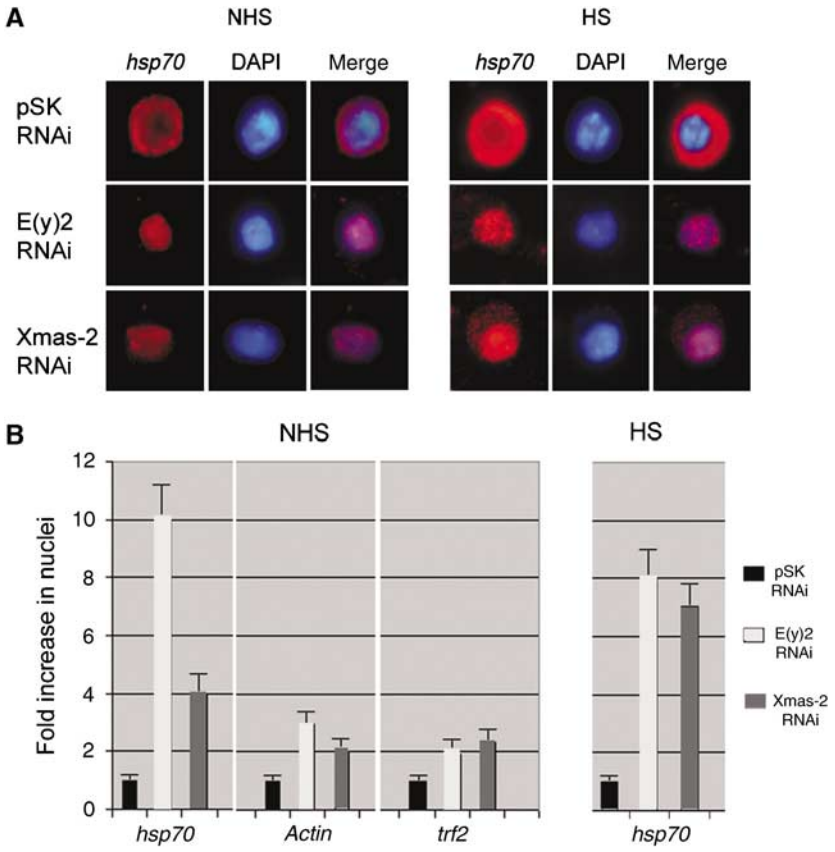

Figure 5 The knockdown of E(y)2 or Xmas-2 expression in S2 cells inhibits the transport of individual transcripts. (**A**) The distribution of *hsp70* transcript was detected by RNA FISH in control (pSK II), E(y)2 or Xmas-2 RNAi-treated S2 cells. The Cy3-labelled probe corresponding to *hsp70* fragment was used to identify *hsp70* transcripts (red). Nuclei are stained with DAPI. Representative examples of cells are shown (magnification, × 1000). (**B**) Quantification of the levels of *hsp70*, *actin* and *trf2* transcripts in the nuclei of control (pSK), E(y)2 or Xmas-2 RNAi-treated S2 cells without HS (NHS) or after HS. Nuclei were isolated, RNA prepared and the individual transcript levels were measured by RT–qPCR using oligonucleotides amplifying the indicated transcripts. In each experiment, the level of transcript present in the control RNAi was taken as one (black bars) and the changes due to the E(y)2 RNAi (light gray bars) or the Xmas-2 RNAi (dark gray bars) are represented as fold changes compared to the control. The error bars represent the variations between three independent experiments.

hsp70 gene cluster remained peripheral, the number of loci close to the nuclear periphery increased only slightly (to 68%) (see WT HS *hsp70* Figure 6Ab and B), and the six genes stayed mainly in one or two big territories. In contrast, the two genomic copies of the control constitutively expressed *trf2* gene were localized as two independent dots and displayed a random distribution (33% of signals were detected at the nuclear periphery), regardless whether the cells were heat shocked or not (see WT NHS and HS *trf2*, Figure 6A, panels c and dand Figure 6B). Interestingly, knockdown of either E(y)2 or Xmas-2 by RNAi under NHS conditions led to the decrease of *hsp70* genes positioned at the nuclear periphery (Figure 6A, panel a), causing almost their random distribution in the nucleus (Figure 6B). Under HS conditions, the E(y)2 or the Xmas-2 RNAi knockdown also destroyed the peripheral localization of the *hsp70* gene cluster (Figure 6A, panel band Figure 6B). Surprisingly, in most of the cases, both under NHS or HS conditions *hsp70* genes left their territory, in which they were clustered together under wild-type conditions, and became individually visible (Figure 6A, panels a and b). In contrast, the control RNAi (pBSK RNAi) had no effect on the positioning of the *hsp70* genes, and similarly E(y)2, Xmas-2 or control knockdown had no effect on the localization of the *trf2* gene either under NHS or HS conditions (Figure 6A, panels a–d and Figure 6B). These results together demonstrate that E(y)2 and Xmas-2 are not only required for the efficient transport of mRNAs, but in addition are also necessary for anchoring of the *hsp70* gene cluster to the nuclear periphery independently whether the *hsp70* genes are activated or not.

E(y)2 and Xmas-2 are also involved in the efficient transcription regulation of the hsp70 loci

As our polytene chromosome labelling experiments demonstrated the presence of E(y)2 and several dSAGA/TFTC subunits before and after HS at the *hsp70* promoter-containing 87A and 87B loci (Supplementary Figure 6; Lebedeva *et al*, 2005), and as E(y)2 interacts with Xmas-2, we investigated the role of E(y)2 and Xmas-2 in the regulation of Pol II transcription initiated from *hsp70* promoters. To this end, we studied the effect of either E(y)2 or Xmas-2 RNAi knockdown on the total *hsp70* mRNA level by real-time PCR (Figure 5C). Without HS in S2 cells we measured low, but detectable level of total *hsp70* transcripts (Figure 5C), and following the RNAi knockdown of E(y)2, the weak constitutive *hsp70* mRNA level was 1.7-fold upregulated as quantified by RT–PCR, whereas Xmas-2 knockdown decreased the *hsp70* RNA level by about 40% (Figure 5C). In contrast, under HS conditions, the knockdown of E(y)2 reduced the strong total *hsp70* mRNA expression levels by 57%

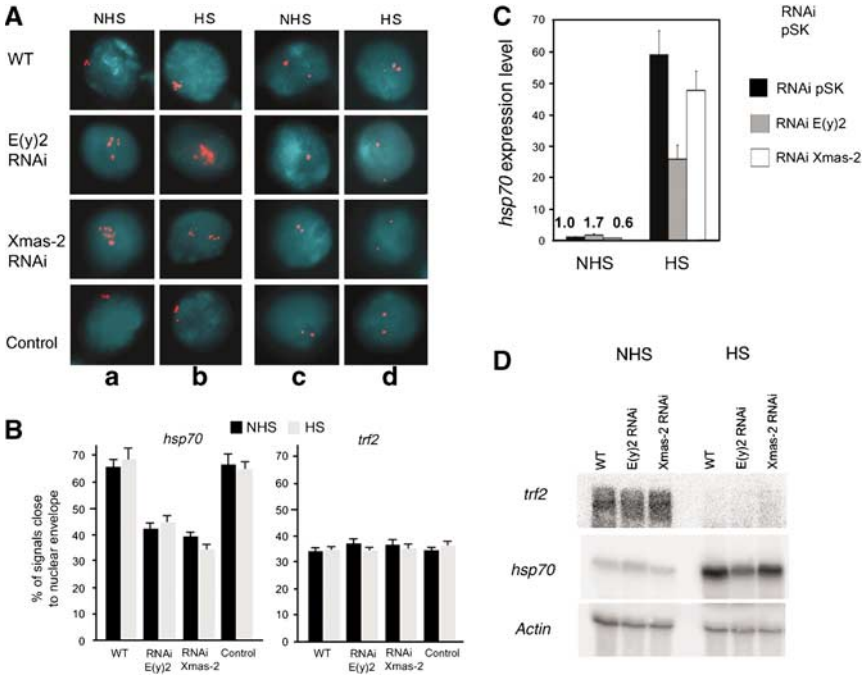

Figure 6 E(y)2 and Xmas-2 participate in the anchoring of the *hsp70* loci to the nuclear envelope and in the regulation of transcription of the *hsp70* gene. (**A**) Localization of the *hsp70* (panels a and b) or the *trf2* (panels c and d) loci in wild-type S2 cells and after E(y)2, Xmas-2 or control RNAi before (panels a and c) and after (panels b and d) HS was revealed by DNA FISH (red). Nuclei are stained with DAPI (blue). The six copies of the *hsp70* genes present at different chromosomal locations (87A and 87B) are grouped as detected by DNA FISH (magnification, $\times$ 1000). (**B**) The quantification of the presence of the *hsp70* and the *trf2* loci at the nuclear periphery in wild-type S2 and RNAi-treated cells as indicated in (A) before and after HS. For each cell, the confocal single-plane image was used to determine the position of the signal relative to the nuclear envelope (see Materials and methods). The nuclear space was divided into three concentric zones of equal surface as described by Taddei *et al* (2006). Bar graphs represent the percent of spots in the zone closest to the nuclear envelope without HS (black bars) and after HS induction (gray bars). Each bar represents the mean value from three different RNAi experiments $\pm$ s.e.m. (**C**) The level of the *hsp70* transcript was measured by RT–PCR in S2 cells after control RNAi (black bars) and after RNAi of E(y)2 (gray bars) and Xmas-2 (empty bars). The values of the *hsp70* transcript without HS are indicated above the bars (the standard deviation is less than 10%). Expression levels in total RNA preparations were normalized against rRNA. The level of *hsp70* transcript in wild-type cells without HS was set to one. (**D**) Northern blot analysis of individual transcripts after RNAi treatment. Total RNA was prepared from control, E(y)2 or Xmas-2 RNAi-treated S2 cells. Probes specific for individual genes (as indicated) were used for hybridization. A 10 μg portion of total RNA was loaded per lane. The 18s rRNA was used as a loading control (data not shown).

(Figure 5C), whereas Xmas-2 RNAi decreased the total *hsp70* mRNA by only 18%. Note that by northern blot analysis we observed the same effects as that by the RT–PCR analysis, and also that due to the RNAi treatment we did not detect any degradation or changes in the quality of the isolated mRNAs (Figure 5D). Thus, under NHS conditions E(y)2 seems to have a negative effect on the weak constitutive *hsp70* transcription, whereas after HS activation it seems to play a positive coactivator role. Interestingly, under NHS and HS conditions Xmas-2 seems to be functioning rather as a positive factor for *hsp70* transcription.

Discussion

Association of the hsp70 loci with the nuclear pore complex is necessary for efficient gene expression and mRNA export

In this study, we have investigated the importance of the nuclear positioning of a gene in its transcription and the consequent export of the nascent mRNA. Our results demonstrate that the E(y)2- and Xmas-2-mediated association of the *hsp70* loci with NPCs is necessary for the regulated and efficient expression of these genes as well as for the mRNA export of their transcripts in *Drosophila* cells. Interestingly, the *hsp70* loci are found at nuclear periphery even before activation, when they show only a basal constitutive activity. Similar to the *hsp70* genes, the metallothienin *MtnA* gene-containing transgene cluster, inducible with heavy metals, localizes to the nuclear periphery before and after induction in S2 cells (Marr *et al*, 2006). These stress-induced *Drosophila* genes appear differentially regulated from the previously described inducible yeast genes, which associate with the nuclear periphery only following activation (Brickner and Walter, 2004; Casolari *et al*, 2004; Cabal *et al*, 2006; Taddei *et al*, 2006). Despite that the *hsp70* loci are kept at the periphery before or after activation, their NPC-anchored positioning is necessary for their full transcriptional activity and the efficient mRNA export.

The comparison of the nuclear localization of several dSAGA/TFTC subunits and E(y)2 suggests that only a subset of dSAGA/TFTC-dependent genes will be anchored to the nuclear periphery for efficient transcription. It is thus conceivable that stress-regulated genes, which seem to be rather SAGA dependent (Huisinga and Pugh, 2004), are anchored to the nuclear periphery. Following stress, this would allow a quick and efficient export of the newly made transcripts through the NPC channel to the cytoplasm and thus a rapid translation of the mRNA. The rapidity and efficiency of this process could be crucial for the survival of the cells under stress conditions.

How are E(y)2 and Xmas-2 linking transcription, NPC-anchoring and export?

E(y)2 is present at the *hsp70* gene-containing loci together with other dSAGA/TFTC subunits, such as dTRRAP and dGCN5, both before and after HS activation (Lebedeva *et al*, 2005; Supplementary Figure 6). The finding that E(y)2 is present at the *hsp70* genes even before the activation is in good agreement with the observation that the *hsp70* loci are anchored to the periphery in a E(y)2-dependent way, but independent of their activation state (Figure 6A and B). It is possible that the position of a given loci is simply dependent on whether E(y)2 (and possibly Xmas-2) is present at the gene before activation (the case of the *Drosophila hsp70* promoters) or actively recruited to promoters by an activator. In agreement with this model, E(y)2 and Xmas-2 downregulation releases the HS loci from the nuclear periphery before and after activation. Thus, E(y)2 and Xmas-2 are key factors in the dynamic process that determines the nuclear positioning and the consequent transcription state of the *hsp70* loci and possibly a subset of other E(y)2-Xmas-2-regulated genes. Moreover, both E(y)2 and Xmas-2 seem to be components of a novel *Drosophila* AMEX.

E(y)2 is present in two functionally different *Drosophila* multiprotein complexes: the SAGA/TFTC-type HAT complex and the AMEX complex. Whether (i) the two E(y)2-containing complexes (SAGA/TFTC and AMEX) need to contact each other on the *hsp70* promoter to achieve efficient transcription, export and to keep the *hsp70* loci anchored at the periphery, or whether (ii) E(y)2 is brought to the promoter by SAGA/TFTC complex and only then would E(y)2 bind to the NPC-associated Xmas-2, are both possible scenarios. The second scenario would be in good agreement with our finding that not all the peripheral E(y)2 is associated with Xmas-2. These models need to be further dissected to explain how E(y)2 and Xmas-2 link gene anchoring and transcription regulation to the NPCs and to consequent mRNA export. Importantly, however, the knockdown of E(y)2 and Xmas-2 causes defects in Pol II transcription, anchoring and mRNA export, although to different degrees. E(y)2 and Xmas-2 RNAi have approximately the same effect on the anchoring of the *hsp70* genes to the nuclei, their effect on the level of transcription of the *hsp70* genes seems to be different. These differences could simply come from the fact that the residual Xmas-2 (Figure 4A) can still function in some extent in transcription, in contrary to E(y)2 that seems to be completely missing (Figure 4A). Interestingly, however, the almost complete ablation of E(y)2 reduces the *hsp70* transcription only by 50%. This suggests that 'being at the periphery' is beneficial for *hsp70* transcription, but not absolutely required. In contrast, not 'being at the periphery' may be absolutely required for quick mRNA export. As all these processes (transcription, anchoring and mRNA export) seem to be spatially and functionally imbricated (at least for a subset of stress-regulated genes), it is difficult to determine how SAGA/TFTC, E(y)2 and Xmas-2 affect the individual steps of these three processes.

In many respect, E(y)2 and Xmas-2 resemble their yeast counterparts Sus1 and Sac3 (Rodriguez-Navarro *et al*, 2004), respectively, which are present in the yeast mRNA export complex. The common features between the *Drosophila* and the corresponding yeast factors are: (i) E(y)2 interacts with Xmas-2, (ii) E(2) and Xmas-2 localize to the nuclear periphery and often to NPCs, (iii) the nuclear peripheral localization of E(y)2 depends on Xmas-2 and (iv) the knockdown of E(y)2 or Xmas-2 reduces certain gene location in the peripheral volume of *Drosophila* cells. All these observations together show that in *Drosophila* (and probably in all metazoans) an evolutionarily conserved AMEX complex exists. The *Drosophila* AMEX complex is formed by E(y)2, Xmas-2 and probably another factor(s) that still needs to be identified. Our results suggest that AMEX has a general function in mRNA export. The knockdown of either E(y)2 or Xmas-2 expression, leads to the strong accumulation of poly(A)+ RNA in the nucleus. This result was also confirmed for individual transcripts. Importantly, the components of AMEX complex are essential for mRNA export both in normal and stress conditions.

Our results argue that E(y)2 interacts with NPCs through the AMEX complex. In our attempt to find E(y)2-interacting proteins by yeast two-hybrid screen, we did not find any nucleoporin (data not shown). It is more likely that Xmas-2 may be implicated in the direct interaction with NPCs as Xmas-2 RNAi interferes the peripheral positioning of E(y)2 (Supplementary Figure 2). Interestingly, in rare cases, E(y)2 labelling was also detected on the cytoplasmic side of the NPCs (Figure 2D, left panel), in agreement with results in yeast, where a component of AMEX (ySac3) has been detected at cytoplasmic fibrils of NPCs (Lei *et al*, 2003).

The fact that synthetic lethality exists between components of the Thp1–Sac3-Sus1p (AMEX) complex and another evolutionarily conserved mRNA export complex, the TREX complex (see Introduction), suggests that these complexes participate in parallel pathways linking transcription to the export machinery (Fischer *et al*, 2002). However, it is also possible that the TREX and the AMEX complexes are sequentially recruited to the same transcript to transport the mRNA to the NPC. As the TREX complex was shown to be cotranscriptionally recruited to the nascent RNA in yeast and during splicing in human cells (Reed and Cheng 2005), it is conceivable that the *Drosophila* TREX will be the first to bind to the *hsp70* transcripts and then subsequently bind to, or handle over the mRNA to, the AMEX complex for efficient mRNA export.

In this study, we demonstrate that in metazoan cells the dSAGA/TFTC complex functions together with the evolutionarily conserved mRNA complex, AMEX, in the anchoring of a subset of transcription sites to the NPC basket to achieve efficient transcription and the consequent mRNA export. Further experiments will be necessary to analyze systematically those metazoan genes for which the transcription-associated NPC anchoring is necessary to achieve full expression and the consequent quick mRNA export through the NPCs.

Materials and methods

Yeast two-hybrid assay

E(y)2 fused to the C-terminus of LexA in pBTM117c vector was used as a bait to screen the library of 1×10^7 cDNA clones fused to GAL4-activating domain in pACT2 vector. Lc40c yeast strain was used for this experiment (Wanker *et al*, 1997). Activation of the LacZ reporter gene was assayed using CPRG as a substrate. The false positives were eliminated by standard procedure (Clontech).

Expression vectors

Xmas-2 cDNA (amino acids 755–1370) and TRF2 cDNA (amino acids 2–442) were fused to three N-terminal FLAG epitopes and cloned into the pAC5.1v5His expression vector (Invitrogen).

RNA interference experiments

RNAi experiments were performed as described previously (Clemens *et al*, 2000). The E(y)2 mRNA was synthesized using Ambion MEGA script T7 kit. The following primers were used to amplify the different dsRNAs: E(y)2 dsRNA 5′-GAATTAATACGACTCACTA TAGGG-AGCACTTCCGGCGCAGTTGATC and 5′-GAATTAATACGACT CACTATAGG-GAGGATTCGTCCTCTGGCTCA, Xmas-2 dsRNA 5′-GAA TTAATACGACTCACTATAGGGAGAATGACCTGCACCGTAAG and GA ATTAATACGACTCACTATAGGGAGACCGGTTGTAGTTCATAG. The fragment of pBluescript II—(pBSK-) vector was used as a negative control: 5′-GAATTAATACGACTCACTATAGGGAGAGTTACATGATCCCCCATG and 5′-GAATTAATACGACTCACTATAGGGAGATTTCGCCCCGAAGAACG.

Antibodies

The rabbit and mouse antibodies against E(y)2 (Georgieva *et al*, 2001), TAF10 (Georgieva *et al*, 2000), TRRAP, GCN5 (Lebedeva *et al*, 2005) and ADA2B (Muratoglu *et al*, 2003) were described previously. Xmas-2 rabbit polyclonal antibodies were obtained against a 616-amino-acid Xmas-2 polypeptide (from 755 to 1370 amino acids). Antibody against lamin Dmo that was developed by PA Fisher was from Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biological Sciences. Mab414 was from Abcam and antibody against the FLAG was from Sigma. All rabbit antibodies used for immunostaining were affinity purified.

Nuclear extracts, immunoprecipitation, immunostaining experiments and microscopy

Preparation of nuclear extracts and co-IP was performed as described previously (Georgieva *et al*, 2001). For preparation of protein extracts from S2 cells, buffer containing 20 mM HEPES, pH7.6, 5 mM $MgCl_2$, 10% glycerol, 0.1 M EDTA, 0.5 M EGTA, 1 mM DTT, 0.3% NP-40 and 0.42 M NaCl, was used. For IP, extracts were diluted by a threefold with the same buffer, but lacking EGTA, DTT, NP-40 and NaCl. Before IP, the extract was treated with DNase (USB, 0.6 μg/1.7 ml of extract) and RNase (Stratagene, 20 μg/1.7 ml of extract). The protein A-Sepharose-bound proteins were washed consequently with 500 mM KCl and 100 mM KCl containing IP buffer (Georgieva *et al*, 2001). Fixation and squashing of salivary glands and antibody staining was performed as described previously (Platero *et al*, 1996). Mouse antibodies against E(y)2 and rabbit antibodies against GCN5 or Xmas-2 were used for colocalization. For immunostaining experiments, the S2 cells were grown on coverslips, washed twice with 1 × PBS, fixed in 3.7% PFA for 10 min, permeabilized in 0.2% Triton X-100 for 5 min and blocked with 3% milk/1 × PBS for 10 min. Primary antibodies were diluted at working concentrations in 3% milk/1 × PBS and stained for 60 min at room temperature in a humid chamber. Cy3-conjugated goat anti-rabbit IgG antibody (Amersham) and Alexa Fluor 488-conjugated goat anti-mouse IgG antibody (Molecular Probes) were used as secondary antibodies. After washing cells were mounted in Vectashield (Vector laboratories). The results were examined using a Leica TCS SP2 confocal microscope or a DMR/HC5 fluorescence microscope (Leica) equipped with a HCX PZ Fluotar × 100/1.3 objective and recorded using a CCD DC 350 F camera.

FISH

RNA *in situ* hybridization combined with immunostaining was carried out as reported previously (Herold *et al*, 2001). The Cy3-labelled oligo-dT probe of 50 bp was used. For DNA FISH, S2 cells were fixed as described previously (Denegri *et al*, 2002). The following probes were used: for *hsp70*—PCR, a fragment of 2405 bp (primes: CGACATACTGCTCTCGTTGGTTC and AGCTAAAATCAATT TGTTGCTAACTT); for *trf2*, full-length cDNA (DQ162845) clone in pBlueScriptII SK-. Probes were labelled with biotinylated-16-dUTP, using Biotin-Nick Translation Mix (Roche). The purified labelled probe was resuspended in hybridization buffer (50% formamide, 2 × SSC, 10% Dextran sulfate, 1% Tween-20, 1 μg/μl yeast tRNA and 0.5 μg/μl sonicated ssDNA) at a final concentration of 2–5 ng/μl. A 10 μl volume of the probe was added to the preparation, denatured at 75°C for 5 min and hybridization was performed overnight at 37°C. Washings were performed in 50% formamide, 2 × SSC at 45°C and in 0.1 × SSC at 55°C. Probes were visualized using three layers of TRITC-conjugated avidin (Invitrogen) and biotin-conjugated antiavidin D antibody (Vector Laboratories).

Image analysis

The Leica TCS SP2 confocal microscope was used to capture 3D image z-sections of the preparations with the step size of 120 nm. For each cell, the zonal position of the signal was determined in one focal section. To eliminate the error, which may arise from the small zone width only signals lying in focal plane, which passed close to the center of the nucleus, were counted. Briefly, the nuclear space was divided into three concentric zones of equal surface and the signal was mapped to one of the three zones by dividing spot to pore distance by the nuclear diameter as described previously (Hediger *et al*, 2004; Taddei *et al*, 2004). The number of the nuclei in which the signal was mapped in the zone closest to the nuclear periphery was compared to the overall number of the nuclei. Three hundred cells from three independent RNAi experiments were counted.

Northern blot analysis and RT–PCR analysis of gene expression

Total cell RNA was extracted from S2 cell culture with Trizol (Invitrogen) and 10-μg samples were electrophoresed through 0.8% agarose. For RT–PCR analysis, total and nuclear RNA was isolated by using RNeasy Mini Kit (Qiagen). To prepare the nuclear RNA, cells were lysed in buffer: 50 mM Tris–HCl, pH 8.0, 140 mM NaCl, 1.5 mM $MgCl_2$, 0.5% NP-40, 1000 U/ml RNase inhibitor and 1 mM DTT, and the nuclei were collected by centrifugation. Reverse transcription was performed on 2 μg RNA using SuperScript III (Invitrogen) and oligo-dT primer. PCR amplification of cDNA was performed using SYBR Green I on a MiniOpticon instrument (BioRad). Primers used for PCR reaction were as follows: *hsp70* (forward, +1915 AGGTCGACTAAAGCCAAATAGA; reverse, +2066 AGCTAAAATCAATTTGTTGCTAACTT), *E(y)2* (forward, +137 GGA GAAGGGCACCAACAACAG; reverse, +322 TGACGCTGAGTTAAAG TTAGGATTCG), Xmas-2 (forward, +396 AGTTCAATGCTGCCTGC CATAC; reverse +580 GCTTGTGTTGCGTTCGGTT), trf2 (forward, GGCGACACATCTGCGTAATCCTCTC; reverse, TGGTCTGTTGCTGCT GCGGTTG), actin (forward, GGCACCACACCTTCTACAATGAGC; reverse, GAGGCGTACAGCGAGAGCACAG) where +1 is the first nucleotide of the coding region of the gene.

Electron microscopy

Ultrathin cryosections of *Drosophila* S2 cells were immunolabelled according to previously described methods (Tokuyasu, 1980). Briefly, cells were fixed with 2.5% paraformaldehyde and 0.1% glutaraldehyde for 1 h. The free aldehydes were blocked with 0.2% glycine in phosphate buffer. The cells were then embedded in 10% gelatin and immediately pelleted. Gelatin-embedded cell pellets were sectioned into cubes deposited in 2.3 M ice-cold sucrose and agitated in a cold room for at least 2 h. Each cube was mounted on a pin and rapidly frozen in liquid nitrogen. The 100 nm thin sections were cut with a cryo-immuno diamond knife (Diatome) using a cryo-ultramicrotome (UM6-Leica), deposited on nickel formvar-carbon-coated 50 hexagonal mesh grids and incubated over droplets of purified E(y)2 antibody (diluted at 1:1000, through 1:200) using an automatic labelling apparatus (IGL, Leica). Primary antibodies were detected using protein A coupled to 10 nm colloidal gold. Sections were embedded and stained with methylcellulose-uranyl acetate and observed using a Philips CM 120 transmission electron microscope at an accelerating voltage of 80 kV.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (http://www.embojournal.org).

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References

Akhtar A, Gasser SM (2007) The nuclear envelope and transcriptional control. *Nat Rev Genet* **8:** 507–517

Brickner JH, Walter P (2004) Gene recruitment of the activated INO1 locus to the nuclear membrane. *PLoS Biol* **2:** e342

Cabal GG, Genovesio A, Rodriguez-Navarro S, Zimmer C, Gadal O, Lesne A, Buc H, Feuerbach-Fournier F, Olivo-Marin JC, Hurt EC, Nehrbass U (2006) SAGA interacting factors confine sub-diffusion of transcribed genes to the nuclear envelope. *Nature* **441:** 770–773

Casolari JM, Brown CR, Komili S, West J, Hieronymus H, Silver PA (2004) Genome-wide localization of the nuclear transport machinery couples transcriptional status and nuclear organization. *Cell* **117:** 427–439

Clemens JC, Worby CA, Simonson-Leff N, Muda M, Maehama T, Hemmings BA, Dixon JE (2000) Use of double-stranded RNA interference in *Drosophila* cell lines to dissect signal transduction pathways. *Proc Natl Acad Sci USA* **97:** 6499–6503

Cockell M, Gasser SM (1999) Nuclear compartments and gene regulation. *Curr Opin Genet Dev* **9:** 199–205

Dantonel JC, Murthy KG, Manley JL, Tora L (1997) Transcription factor TFIID recruits factor CPSF for formation of 3′ end of mRNA. *Nature* **389:** 399–402

Denegri M, Moralli D, Rocchi M, Biggiogera M, Raimondi E, Cobianchi F, De Carli L, Riva S, Biamonti G (2002) Human chromosomes 9, 12, and 15 contain the nucleation sites of stress-induced nuclear bodies. *Mol Biol Cell* **13:** 2069–2079

Fischer T, Strasser K, Racz A, Rodriguez-Navarro S, Oppizzi M, Ihrig P, Lechner J, Hurt E (2002) The mRNA export machinery requires the novel Sac3p-Thp1p complex to dock at the nucleoplasmic entrance of the nuclear pores. *EMBO J* **21:** 5843–5852

Gallardo M, Aguilera A (2001) A new hyperrecombination mutation identifies a novel yeast gene, THP1, connecting transcription elongation with mitotic recombination. *Genetics* **157:** 79–89

Georgiev PG (1994) Identification of mutations in three genes that interact with zeste in the control of white gene expression in *Drosophila melanogaster*. *Genetics* **138:** 733–739

Georgieva S, Kirschner DB, Jagla T, Nabirochkina E, Hanke S, Schenkel H, de Lorenzo C, Sinha P, Jagla K, Mechler B, Tora L (2000) Two novel *Drosophila* TAF(II)s have homology with human TAF(II)30 and are differentially regulated during development. *Mol Cell Biol* **20:** 1639–1648

Georgieva S, Nabirochkina E, Dilworth FJ, Eickhoff H, Becker P, Tora L, Georgiev P, Soldatov A (2001) The novel transcription factor e(y)2 interacts with TAF(II)40 and potentiates transcription activation on chromatin templates. *Mol Cell Biol* **21:** 5223–5231

Hediger F, Taddei A, Neumann FR, Gasser SM (2004) Methods for visualizing chromatin dynamics in living yeast. *Methods Enzymol* **375:** 345–365

Herold A, Klymenko T, Izaurralde E (2001) NXF1/p15 heterodimers are essential for mRNA nuclear export in *Drosophila*. *RNA* **7:** 1768–1780

Huisinga KL, Pugh BF (2004) A genome-wide housekeeping role for TFIID and a highly regulated stress-related role for SAGA in *Saccharomyces cerevisiae*. *Mol Cell* **13:** 573–585

Ishii K, Arib G, Lin C, Van Houwe G, Laemmli UK (2002) Chromatin boundaries in budding yeast: the nuclear pore connection. *Cell* **109:** 551–562

Kohler A, Pascual-Garcia P, Llopis A, Zapater M, Posas F, Hurt E, Rodriguez-Navarro S (2006) The mRNA export factor Sus1 is involved in SAGA-mediated H2B deubiquitinylation through its interaction with Ubp8 and Sgf11. *Mol Biol Cell* **17:** 4228–4236

Kusch T, Guelman S, Abmayr SM, Workman JL (2003) Two *Drosophila* Ada2 homologues function in different multiprotein complexes. *Mol Cell Biol* **23:** 3305–3319

Lebedeva LA, Nabirochkina EN, Kurshakova MM, Robert F, Krasnov AN, Evgen'ev MB, Kadonaga JT, Georgieva SG, Tora L (2005) Occupancy of the *Drosophila* hsp70 promoter by a subset of basal transcription factors diminishes upon transcriptional activation. *Proc Natl Acad Sci USA* **102:** 18087–18092

Lei EP, Stern CA, Fahrenkrog B, Moy TI, Aebi U, Silver PA (2003) Sac3 is an mRNA export factor that localizes to cytoplasmic fibrils of nuclear pore complex. *Mol Biol Cell* **14:** 836–847

Manley JL (2002) Nuclear coupling: RNA processing reaches back to transcription. *Nat Struct Biol* **9:** 790–791

Marr II MT, Isogai Y, Wright KJ, Tjian R (2006) Coactivator cross-talk specifies transcriptional output. *Genes Dev* **20:** 1458–1469

Muratoglu S, Georgieva S, Papai G, Scheer E, Enunlu I, Komonyi O, Cserpan I, Lebedeva L, Nabirochkina E, Udvardy A, Tora L, Boros I (2003) Two different *Drosophila* ADA2 homologues are present in distinct GCN5 histone acetyltransferase-containing complexes. *Mol Cell Biol* **23:** 306–321

Pankotai T, Komonyi O, Bodai L, Ujfaludi Z, Muratoglu S, Ciurciu A, Tora L, Szabad J, Boros I (2005) The homologous *Drosophila* transcriptional adaptors ADA2a and ADA2b are both required for normal development but have different functions. *Mol Cell Biol* **25:** 8215–8227

Pickersgill H, Kalverda B, de Wit E, Talhout W, Fornerod M, van Steensel B (2006) Characterization of the *Drosophila melanogaster* genome at the nuclear lamina. *Nat Genet* **38:** 1005–1014

Platero JS, Sharp EJ, Adler PN, Eissenberg JC (1996) *In vivo* assay for protein-protein interactions using *Drosophila* chromosomes. *Chromosoma* **104:** 393–404

Proudfoot NJ, Furger A, Dye MJ (2002) Integrating mRNA processing with transcription. *Cell* **108:** 501–512

Reed R, Cheng H (2005) TREX, SR proteins and export of mRNA. *Curr Opin Cell Biol* **17:** 269–273

Rodriguez-Navarro S, Fischer T, Luo MJ, Antunez O, Brettschneider S, Lechner J, Perez-Ortin JE, Reed R, Hurt E (2004) Sus1, a functional component of the SAGA histone acetylase complex and the nuclear pore-associated mRNA export machinery. *Cell* **116:** 75–86

Schmid M, Arib G, Laemmli C, Nishikawa J, Durussel T, Laemmli UK (2006) Nup-PI: the nucleopore–promoter interaction of genes in yeast. *Mol Cell* **21:** 379–391

Taddei A, Hediger F, Neumann FR, Bauer C, Gasser SM (2004) Separation of silencing from perinuclear anchoring functions in yeast Ku80, Sir4 and Esc1 proteins. *EMBO J* **23:** 1301–1312

Taddei A, Van Houwe G, Hediger F, Kalck V, Cubizolles F, Schober H, Gasser SM (2006) Nuclear pore association confers optimal expression levels for an inducible yeast gene. *Nature* **441:** 774–778

Tokuyasu KT (1980) Immunochemistry on ultrathin frozen sections. *Histochem J* **12:** 381–403

Vinciguerra P, Stutz F (2004) mRNA export: an assembly line from genes to nuclear pores. *Curr Opin Cell Biol* **16:** 285–292

Wanker EE, Rovira C, Scherzinger E, Hasenbank R, Walter S, Tait D, Colicelli J, Lehrach H (1997) HIP-I: a huntingtin interacting protein isolated by the yeast two-hybrid system. *Hum Mol Genet* **6:** 487–495

Zhimulev IF (1999) Genetic organization of polytene chromosomes. *Adv Genet* **39:** 1–589

Zink D, Amaral MD, Englmann A, Lang S, Clarke LA, Rudolph C, Alt F, Luther K, Braz C, Sadoni N, Rosenecker J, Schindelhauer D (2004) Transcription-dependent spatial arrangements of CFTR and adjacent genes in human cell nuclei. *J Cell Biol* **166:** 815–825